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Original Article

Knockdown of PHGDH potentiates 5-FU cytotoxicity in gastric cancer cells via the Bcl-2/Bax/caspase-3 signaling pathway

Yunli Zhang, Litao Yang, Guangou Dai, Hu Cao

Department of Abdominal Surgery, Zhejiang Cancer Hospital, Hangzhou 310022, Zhejiang Province, China

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Abstract: Gastric cancer (GC) is one of the most common malignancies in the world. Fluorouracil (5-FU) is widely used in the treatment of cancers, but resistance to 5-FU results in the failure of chemotherapy. Phosphoglycerate dehydrogenase (PHGDH) has been reported to play a vital role in the development of 5-FU resistance in cancer cells. However, the exact role of PHGDH and the underlying mechanisms for 5-FU resistance in GC cells remain elusive. In this study, PHGDH expression was much higher in the GC tissues of 5-FU-resistant patients than that in the GC tissues of 5-FU-sensitive patients. Moreover, the expression of PHGDH was obviously increased in BGC823/5-FU cells compared with that in BGC823 cells. 5-FU treatment significantly reduced the viability of BGC823/5-FU cells, in a dose- and time-dependent manner. Furthermore, 5-FU treatment inhibited the proliferation of BGC823/5-FU cells, as evidenced by decreased cell viability and reduced colony-forming ability. The knockdown of PHGDH made possible the inhibitory effect of 5-FU on the proliferation of BGC823/5-FU cells. Furthermore, 5-FU treatment promoted apoptosis of BGC823/5-FU cells, as indicated by increased numbers of TUNEL-positive cells and increased rates of apoptosis. Notably, the promoting effect of 5-FU on the apoptosis of BGC823/5-FU cells was markedly enhanced by PHGDH knockdown. Additionally, 5-FU treatment downregulated Bcl-2 expression and upregulated the expression of Bax and caspase-3, and this effect was remarkably enhanced by PHGDH knockdown. In conclusion, knockdown of PHGDH potentiates 5-FU cytotoxicity in GC cells via the Bcl-2/Bax/caspase-3 signaling pathway.

Keywords: Phosphoglycerate dehydrogenase, fluorouracil, gastric cancer, cytotoxicity

Introduction

Gastric cancer (GC) is a kind of malignant tumor that develops in the lining of the stomach. Globally, GC is the fourth most common type of cancer and the third leading cause of cancer deaths, accounting for approximately 952000 new cases annually [1]. GC is difficult to cure unless it is detected at an early stage. Unfortunately, since GC has no characteristic clinical manifestations in its early stages, most patients with GC are diagnosed at an advanced stage, which is one of the main reasons for its high mortality and poor prognosis [2]. Despite substantial improvements in the diagnosis and treatment of GC in the last decade, the 5-year survival rate of patients with GC remains less than 20% [3]. At present, palliative chemotherapy is widely used for the treatment of GC. Chemotherapy can reduce the tumor size, relieve disease symptoms, and increase survival time [4]. However, most GC cells are resistant to chemotherapy drugs, which leads to the failure of chemotherapy [5]. Thus, developing strategies to improve the chemosensitivity of GC cells has become a hot topic for cancer research.

Phosphoglycerate dehydrogenase (PHGDH) is a key rate-limiting enzyme in the de novo biosynthesis of serine that introduces the glycolytic intermediate 3-phosphoglycerate into serine and glycine [6]. Serine and glycine released in the process provide numerous precursor molecules and other substrates required for the growth and proliferation of cancer cells [7]. Recently, more and more attention has been paid to the role of PHGDH in tumor progression. PHGDH expression has been reported to be upregulated in a number of human cancers, such as epithelioid carcinoma [8], glioma [9], melanoma [10], and breast cancer [11]. Downregulation of PHGDH has been shown to strikingly suppress the proliferation, migration, and

invasion abilities of pancreatic cancer cells by inhibiting the expression of cyclin B1, cyclin D1, matrix matalloproteinases-2 and matrix matalloproteinases-9 [12]. Knockdown of PHGDH in breast cancer cells leads to increased oxidant levels, increased apoptosis and loss of breast cancer stem cell enrichment under hypoxic conditions [13]. Notably, PHGDH expression has been negatively correlated with the 5-year survival rate of patients with GC [14]. These observations suggest that PHGDH may be a new target for cancer therapy.

Fluorouracil, also known as 5-FU, is the most widely used cytotoxic drug in the treatment of human cancers. 5-FU can inhibit the synthesis of DNA by interrupting the action of thymidylate synthase. Furthermore, thymidylate synthase methylates deoxyuridine monophosphate to form thymidine monophosphate (dTMP). 5-FU treatment leads to a scarcity of dTMP, ultimately resulting in cell death. Besides, 5-FU induces cell cycle arrest and inhibits RNA synthesis [15]. In the past decades, 5-FU treatment has been shown to markedly reduce GC mortality. However, the efficacy of 5-FU in patients with GC is limited owing to the development of the 5-FU-resistance. Thus, it is important for us to find new strategies for overcoming the 5-FU-resistance in GC.

In this study, we detected the expression of PHGDH in GC tissues and cell lines (BGC823 and BGC823/5-FU cells). Furthermore, we explored the effects of PHGDH knockdown on the proliferation and apoptosis of BGC823/5-FU cells and its underlying molecular mechanisms.

Materials and methods

Patients' characteristics and clinicopathological features

This study received approval from the Ethics Committee of Zhejiang Cancer Hospital. In all, 29 patients with GC who received 5-FU-based chemotherapy signed an informed consent before participating in the project. The treatment was effective in shrinking the primary tumor and reducing malignant pleural effusions. 5-FU-resistant cases were distinguished when the size of the primary tumor increased, pleural effusions increased, or tumor cells metastasized within 12 months, otherwise defined as 5-FU-sensitive (n = 7). The GC tissue samples were obtained from these patients.

Cell culture and transfection

BGC823 cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and incubated in an RPMI-1640 medium containing 10% fetal bovine serum (Solarbio, Beijing, China), 100 μg/ml streptomycin (Solarbio) and 100 U/ml penicillin (Solarbio) ina 5% CO_2 incubator at 37°C.

A 5-Fu-resistant BGC823 cell line (BGC823/5-Fu) was constructed by screening the parental BGC823 cells ina culture medium containing stepwise increased concentrations of 5-Fu (Solarbio) for 12 months. When they acquired resistance to 1 μM 5-FU, the cells were termed as BGC823/5-Fu cells and used for this study.

To knockdown PHGDH, sh-PHGDH-1 and sh-PHGDH-2 were transfected into BGC823/5-FU cells. Forty-eight hours after transfection, BGC-823/5-FU cells were treated with indicated concentrations (0, 1, 2, 4, 8, 16, and 32 μM) of 5-FU for 48 h or exposed to 16 μM 5-FU for different durations (24, 48, and 72 h).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the tissues and cells using TRIzol reagent (Sigma-Aldrich, Shanghai, China) and reverse transcribed to cDNA using cDNA synthesis kit (Roche, Penzberg Germany). PHGDH expression was detected using the ABI Q6 qRT-PCR system (Applied Biosystems Inc, Foster City, CA, USA). The primer sequences used in this study were as follows: PHGDH, forward: 5'-CACATTCTTGGGCTGAAC-3', reverse: 5'-TTATTAGACGGTTATTGCTGTA-3'; β-actin forward: 5'-GTCATTCCAAATATGAGATGCGT-3', reverse: 5'-GCATTACATAATTTACACGAAAGCA-3'. The relative expression level of PHGDH was normalized to that of β-actin (housekeeping gene) using the $2^{-\Delta\Delta Ct}$ method.

Western blot assay

Proteins were isolated with ice-cold RIPA buffer and the protein concentrations were quantified using a BCA protein assay kit (Pierce, Rockford, IL, USA). Cell lysates containing 30 μg of total protein were separated on 10% sodium dodecyl sulfate-polyacrylamide gels, and then transferred to polyvinylidene fluoridemembranes (Millipore Corporation, Billerica,

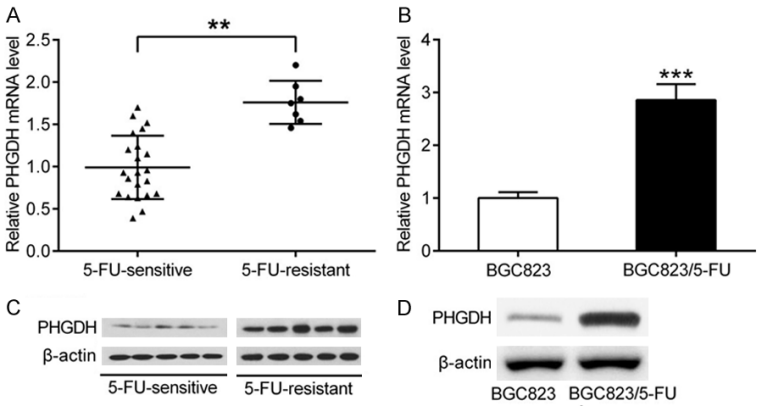

Figure 1. PHGDH expression is significantly upregulated in 5-FU-resistant GC tissues and cells. A. The mRNA expression of PHGDH in the GC tissues of 5-FU-sensitive (n = 22) and 5-FU-resistant patients (n = 7). B. The mRNA expression of PHGDH in BGC823 and BGC823/5-FU cells. C. The protein expression of PHGDH in the GC tissues of 5-FU-sensitive (n = 22) and 5-FU-resistant patients (n = 7). D. The protein expression of PHGDH in BGC823 and BGC823/5-FU cells. $^{**}P<0.01$, $^{***}P<0.001$.

MA, USA). Membranes were blocked with 5% skim milk, immunoblotted overnight at 4°C with primary antibodies against PHGDH (Novus Biologicals, Littleton, CO, USA), Bcl-2 (Abcam, Cambridge, MA, USA), Bax (Abcam), caspase-3 (Abcam), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and then incubated with horseradish peroxidase-conjugated secondary antibody (Boster, Wuhan, China) for 2 h at 25°C. Immunoreactivity was detected using ECL (Pierce) and the intensity of the protein bands was quantified with the Image J software.

Cell counting kit-8 (CCK-8) assay

The viability of BGC823/5-FU cells was determined using the CCK-8 assay (Solarbio) in accordance with the manufacturer's instructions. At 48 h after transfection, BGC823/5-FU cells were treated with indicated concentrations (0, 1, 2, 4, 8, 16, and 32 μM) of 5-FU for 48 h or exposed to 16 μM 5-FU for different durations (24, 48, and 72 h). CCK-8 (10 μl) was added to each well, followed by incubation in a 5% CO_2 incubator at 37°C. The absorbance was measured at 450 nm using a microplate reader (Thermo Scientific, Waltham, MA, USA).

Colony formation assay

The colony-forming ability of BGC823/5-FU cells was evaluated using a colony formation assay. At 48 h after transfection, BGC823/5-FU cells were seeded in a 6-well plate and incubated with 2 μM 5-FU. After 2 weeks of incubation, the clones were fixed in methanol for 20 min, followed by staining with 0.1% crystal violet for 30 min at room temperature. The number of cell colonies with a diameter larger than 0.1 mm was counted under an optical microscope (Leica Microsystems, Wetzlar, Germany).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

A TUNEL assay was carried out to assess cell apoptosis in BGC823/5-FU cells using an In Situ Cell Death Detection Kit (Roche). At 48 h after transfection, BGC823/5-FU cells were incubated with 16 μM 5-FU for 48 h. BGC823/5-FU cells were fixed for 1 h in 4% paraformaldehyde, followed by permeation with 0.1% Triton X-100 for 6 min. Then, BGC823/5-FU cells were stained with the TUNEL reaction mixture for 1 h at 37°C. After washing with phosphate buffered saline (PBS), the cells were incubated with 4',6-diamidino-2-phenylindole (DAPI; Solarbio) for 5 min at room temperature in the dark. The percentage of TUNEL positive cells was quantified using a confocal microscope (Leika, Germany).

Flow cytometry

Apoptosis of BGC823/5-FU cells was evaluated by flow cytometry using an Annexin V-FITC/propidium iodide (PI) apoptosis detection kit (Solarbio). In brief, BGC823/5-FU cells transfected with sh-PHGDH were incubated with 16 μM 5-FU for 48 h. BGC823/5-FU cells were rinsed with PBS and resuspended in a binding buffer (1×). Subsequently, BGC823/5-FU cells were stained for 10 min with Annexin V-FITC and incubated for 5 min with PI in the dark at room temperature. After resuspending in PBS, cells were analyzed by flow cytometry.

Statistical analysis

All data were presented as the mean ± standard deviation (SD). The Student's t-test or one-

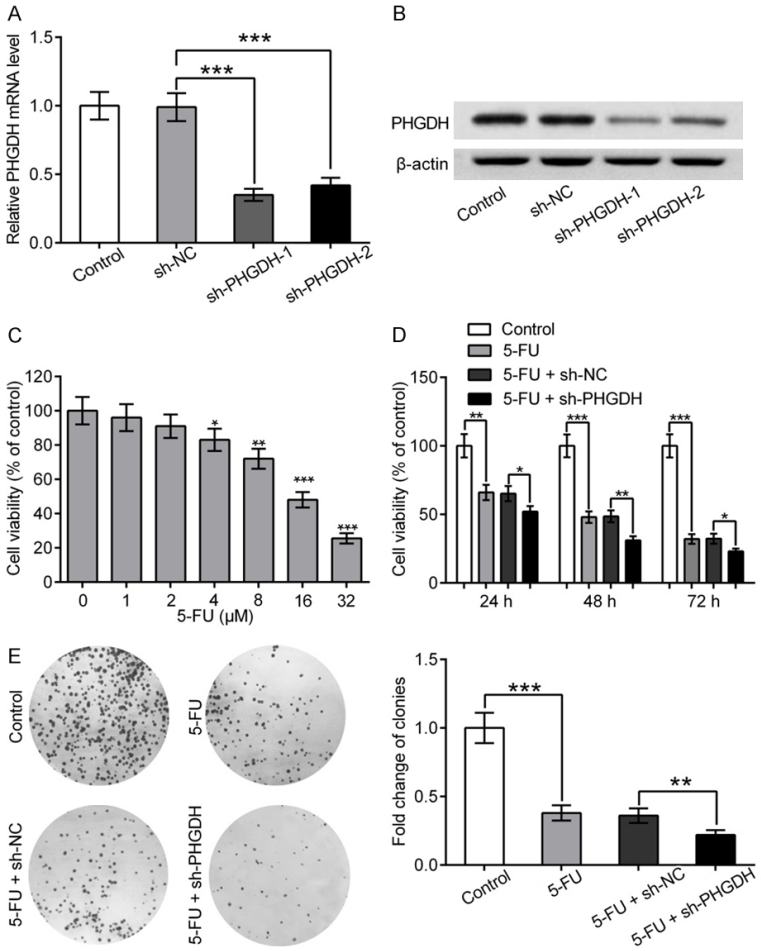

Figure 2. Knockdown of PHGDH inhibits the proliferation of BGC823/5-FU cells. A and B. sh-PHGDH-1, sh-PHGDH-2 or sh-NC plasmid was transfected into BGC823/5-FU cells. The expression of PHGDH was determined by RT-qPCR and western blot analysis. C. BGC823/5-FU cells were incubated with indicated concentrations (0, 1, 2, 4, 8, 16, and 32 μM) of 5-FU for 48 h, and the viability of BGC823/5-FU cells was measured by a CCK-8 assay. D. At 48 h after transfection with sh-PHGDH-1, BGC823/5-FU cells were exposed to 16 μM 5-FU for different durations (24, 48, and 72 h) and their viability was measured by a CCK-8 assay. E. After 2 weeks of treatment with 2 μM 5-FU, the colony-forming ability of BGC823/5-FU cells was analyzed by a colony formation assay. **P*<0.05, ***P*<0.01, ****P*<0.001.

way analysis of variance was utilized for statistical analyses of the data. All statistical analyses were performed using SPSS statistical software version 20.0 (SPSS Inc, Chicago, IL, USA). A *P*-value of 0.05 or less was considered statistically significant.

Results

PHGDH is significantly upregulated in 5-FU-resistant GC tissues and cells

To investigate the role of PHGDH in the development of 5-FU resistance in GC cells, the mRNA and protein expression levels of PHGDH in the GC tissues of 5-FU-sensitive (*n* = 22) and 5-FU-resistant patients (*n* = 7) were determined by RT-qPCR and Western blot assays. Results showed that the mRNA and protein expression levels of PHGDH were much higher in the GC tissues of 5-FU-resistant patients than that in the GC tissues of 5-FU-sensitive patients (**Figure 1A** and **1C**). To confirm the differential expression of PHGDH, we determined the mRNA and protein levels of PHGDH in BGC823 and BGC823/5-FU cells. Compared with the BGC823 cells, the mRNA and protein levels were markedly increased in the BGC823/5-FU cells (**Figure 1B** and **1D**). Therefore, these findings suggest that PHGDH expression is markedly upregulated in 5-FU-resistant GC tissues and cells.

Knockdown of PHGDH inhibits the proliferation of BGC823/5-FU cells

To investigate the effect of PHGDH on the proliferation of BGC823/5-FU cells, we performed loss-of-function experiments by transfecting BGC-823/5-FU cells with sh-PHGDH-1, sh-PHGDH-2, or sh-NC. As illustrated in **Figure 1A** and **1B**, the inhibitory effects of sh-PHGDH-1 and sh-PHGDH-2 on PHGDH mRNA and protein levels were validated by RT-qPCR and western blot analysis, respectively. The mRNA and protein levels of PHGDH were much higher in the sh-NC group than those in the sh-PHGDH group, especially in the sh-PHGDH-1 group (**Figure 2A** and **2B**). Therefore, the sh-PHGDH-1 plasmid was selected for the following experiments. At 48 h after transfection, BGC823/5-FU cells were incubated with the indicated concentrations (0, 1, 2, 4, 8, 16, and 32 μM) of 5-FU for 48 h or treated with 16 μM 5-FU for different duration (24, 48, and 72 h). As detected by the CCK-8 assay, the 5-FU treat-

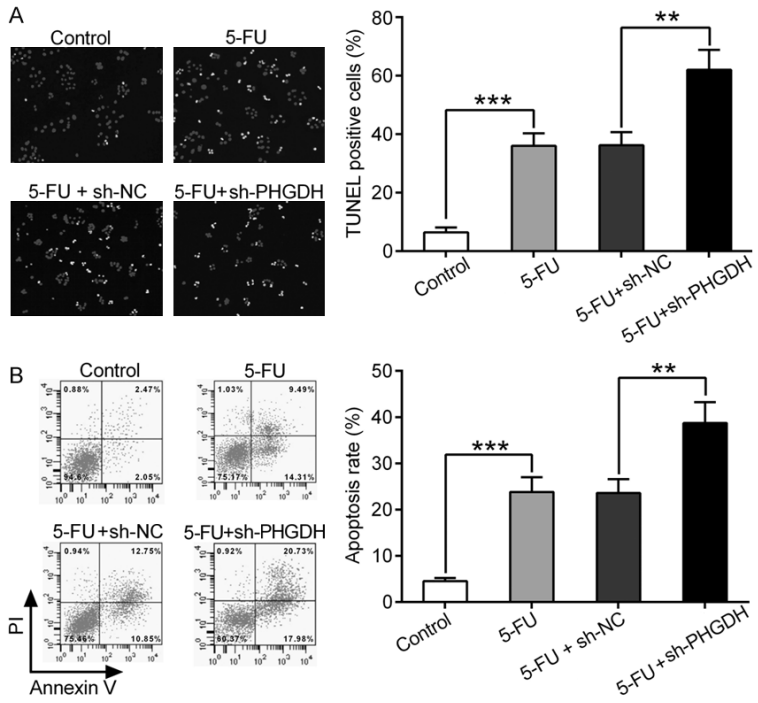

Figure 3. Knockdown of PHGDH promotes apoptosis in BGC823/5-FU cells. TUNEL assay (A) and flow cytometry (B) were carried out in BGC823/5-FU cells transfected with sh-PHGDH-1, following exposure to 16 μM 5-FU for 48 h. $^{**}P<0.01$, $^{***}P<0.001$.

ment significantly reduced the viability of BGC-823/5-FU cells in a concentration-dependent manner (**Figure 2C**). Thus, a dose of 16 μM of 5-FU was chosen for the following experiments. Furthermore, the viability of BGC823/5-FU cells was markedly reduced at 48 and 72 h post 5-FU treatment (**Figure 2D**). Simultaneously, a colony formation assay was performed on BGC823/5-FU cells transfected with sh-PHGDH-1, following exposure to 2 μM 5-FU for 2 weeks. The 5-FU treatment obviously decreased the colony-forming ability of the BGC-823/5-FU cells, which was significantly enhanced by the knockdown of PHGDH (**Figure 2E**). Collectively, these results indicated that the knockdown of PHGDH inhibited the proliferation of the BGC823/5-FU cells.

Knockdown of PHGDH promotes apoptosis of BGC823/5-FU cells

To explore the effect of PHGDH knockdown on the apoptosis of BGC823/5-FU cells, we measured the number of apoptotic BGC823/5-FU cells using the TUNEL assay and flow cytometry. At 48 h post transfection, BGC823/5-FU cells were treated with 16 μM 5-FU for 48 h. The results demonstrated that 5-FU treatment promoted apoptosis in BGC823/5-FU cells as evidenced by the increased numbers of TUNEL-positive cells and increased rates of apoptosis. Notably, the promoting effect of 5-FU on the apoptosis of BGC-823/5-FU cells was prominently enhanced by the knockdown of PHGDH (**Figure 3A** and **3B**). Taken together, these results suggest that the knockdown of PHGDH promotes apoptosis in BGC823/5-FU cells.

Knockdown of PHGDH downregulates Bcl-2 expression and upregulates the expression of Bax and caspase-3

To further confirm whether PHGDH participates in 5-FU-mediated apoptosis by modulating the Bcl-2/Bax/caspase-3 signaling pathway, we performed RT-qPCR and Western blot assays to analyze the expression of Bcl-2, Bax, and caspase-3 in BGC823/5-FU cells transfected with sh-PHGDH-1, following exposure to 16 μM 5-FU for 48 h. 5-FU treatment significantly downregulated the mRNA and protein levels of Bcl-2 and upregulated those of Bax and caspase-3, and this effect was markedly potentiated by the knockdown of PHGDH (**Figure 4A-D**). Taken together, our findings revealed that the knockdown of PHGDH downregulated Bcl-2 expression and upregulated the expression of Bax and caspase-3.

Discussion

Important findings in the last decade have revealed that metabolic reprogramming may play an essential role in the occurrence and development of human cancers [16]. Tumor growth is maintained via aerobic glycolysis, also called the Warburg affect [17]. Moreover, metabolic reprogramming promotes the accumulation of biomass and the growth of cancer cells through modifications in the genomes of metabolic enzymes [18]. Conversely, the inhibition of metabolic enzymes can repress the growth of

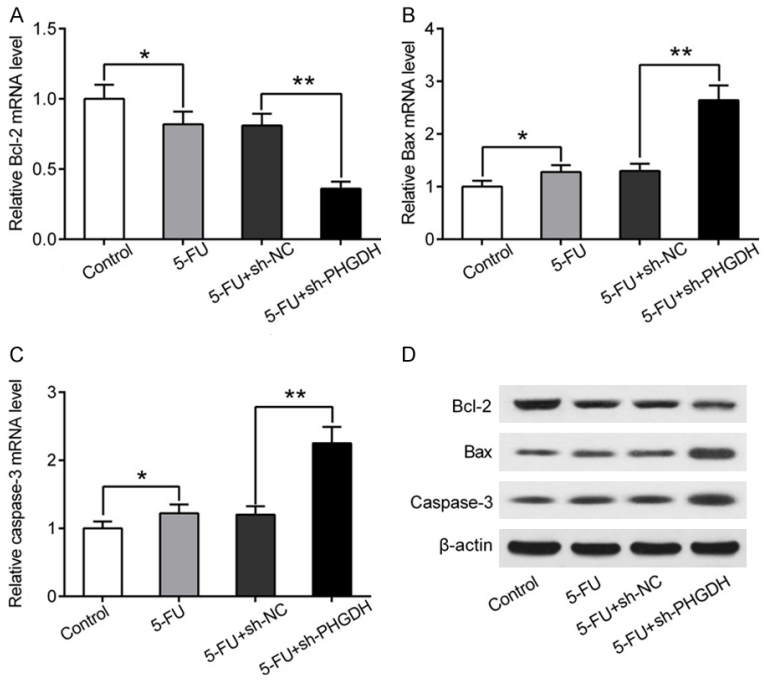

Figure 4. Knockdown of PHGDH downregulates Bcl-2 expression and upregulates the expression of Bax and caspase-3. At 48 h after transfection with sh-PHGDH-1, BGC823/5-FU cells were exposed to 16 μM 5-FU for 48 h. The mRNA expression levels of Bcl-2 (A), Bax (B), and caspase-3 (C) were examined by RT-qPCR. (D) The protein levels of Bcl-2, Bax, and caspase-3 were detected by western blot analysis. **P*<0.05, ***P*<0.01.

cancer cells. Therefore, research on metabolic enzymes holds great promise.

Resistance to chemotherapy drugs often occurs during the treatment of cancers and can be attributed to most cancer-related deaths. Growing evidence suggests that PHGDH plays a crucial role in the development of drug resistance. For instance, Zaal and colleagues demonstrated that PHGDH upregulated inbortezomib (BTZ)-resistant cell lines (BTZ/7 and BTZ/100 cells). Moreover, BTZ resistance is closely correlated with the overexpression of PHGDH [19]. Zhang and colleagues suggested that the inhibition of PHGDH caused doxorubicin-induced oxidative stress and increased the sensitivity of triple-negative breast cancer cells to doxorubicin [11]. Jing and colleagues found that PHGDH was highly expressed in cervical adenocarcinoma, which was associated with tumor size and prognosis. Knockdown of PHGDH in HeLa cells obviously suppressed cell proliferation and enhanced their sensitivity to cisplatin through the downregulation of Bcl-2 and the upregulation of cleaved caspase-3 [20]. However, the role of PHGDH in the development of 5-FU-resistance in GC cells remains unclear. In our study, the expression of PHGDH was much higher in the GC tissues of 5-FU-resistant patients than in the GC tissues of 5-FU-sensitive patients. Furthermore, the expression of PHGDH was strikingly increased in BGC823/5-FU cells compared with that in the BGC823 cells. In addition, 5-FU treatment inhibited the proliferation of BGC823/5-FU cells, as evidenced by decreased cell viability and reduced colony-forming ability. Notably, the knockdown of PHGDH made possible the inhibitory effect of 5-FU on the proliferation of BGC823/5-FU cells.

Apoptosis is a genetically coded program of cell death that maintains homeostasis, which is strictly regulated by polygenes [21]. Chemotherapeutic agents exert their curative effect by inducing apoptosis in cancer cells. Thus, the induction of apoptosis in cancer cells has become a hotspot for the treatment of cancer [22]. Under normal conditions, the pro- and anti-apoptotic proteins are metastable in organisms [23]. The Bcl-2 protein family is a core member of the apoptosis gene family, which is regarded as the primary modulator of cell apoptosis [24]. Bax, a pro-apoptotic protein, functions as an apoptotic activator and induces the opening of the mitochondrial voltage-dependent anion channel, which results in the release of cytochrome c and activation of caspase [25]. Bcl-2, an anti-apoptotic protein, can restrain cell apoptosis by binding pro-apoptotic proteins, such as Bax [26]. Caspase-3, the main executioner caspase in apoptosis, is activated by proteolytic cleavage, which in turn induces apoptosis [27]. In this study, the results of a TUNEL assay and flow cytometry revealed that 5-FU treatment markedly increased BGC823/5-FU cell apoptosis, which was prominently enhanced by the knockdown of PHGDH. Additionally, 5-FU treatment strikingly down-

regulated the mRNA and protein levels of Bcl-2 and upregulated those of Bax and caspase-3, and this effect was markedly potentiated by knockdown of PHGDH.

Taken together, our results suggest that PHGDH expression is upregulated in 5-FU-resistant tissues and cells. The knockdown of PHGDH represses cell proliferation, promotes cell apoptosis, and restores 5-FU sensitivity in BGC823/5-FU cells. Moreover, the knockdown of PHGDH downregulates Bcl-2 expression and upregulates the expression of Bax and caspase-3. Therefore, these findings suggest that 5-FU resistance in GC cells might be combated by targeting PHGDH, which may serve as a novel therapeutic target for GC.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yunli Zhang, Department of Abdominal Surgery, Zhejiang Cancer Hospital, 1 Banshan East Road, Hangzhou 310022, Zhejiang Province, China. Tel: 86-571-88128031; E-mail: zhang_yunli@sohu.com

References

[1] Alexandrov LB, Nik-Zainal S, Siu HC, Leung SY and Stratton MR. A mutational signature in gastric cancer suggests therapeutic strategies. Nat Commun 2015; 6: 8683.

[2] Wadhwa R, Taketa T, Sudo K, Blum MA and Ajani JA. Modern oncological approaches to gastric adenocarcinoma. Gastroenterol Clin North Am 2013; 42: 359-369.

[3] Saeidi H, Nasiri MR, Shahidsales S, Kermani AT, Hematti S, Roodbari SS, Shahri MH and Chagharvand S. Evaluation of estrogen receptor expression and its relationship with clinicopathologic findings in gastric cancer. Adv Biomed Res 2015; 4: 177.

[4] Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R and Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res 2018; 10: 239-248.

[5] Marin JJ, Al-Abdulla R, Lozano E, Briz O, Bujanda L, Banales JM and Macias RI. Mechanisms of resistance to chemotherapy in gastric cancer. Anticancer Agents Med Chem 2016; 16: 318-334.

[6] Zogg CK. Phosphoglycerate dehydrogenase: potential therapeutic target and putative metabolic oncogene. J Oncol 2014; 2014: 524101.

[7] Amelio I, Cutruzzola F, Antonov A, Agostini M and Melino G. Serine and glycine metabolism in cancer. Trends Biochem Sci 2014; 39: 191-198.

[8] Cho HM, Jun DY, Bae MA, Ahn JD and Kim YH. Nucleotide sequence and differential expression of the human 3-phosphoglycerate dehydrogenase gene. Gene 2000; 245: 193-201.

[9] Liu J, Guo S, Li Q, Yang L, Xia Z, Zhang L, Huang Z and Zhang N. Phosphoglycerate dehydrogenase induces glioma cells proliferation and invasion by stabilizing forkhead box M1. J Neurooncol 2013; 111: 245-255.

[10] Ou Y, Wang SJ, Jiang L, Zheng B and Gu W. p53 Protein-mediated regulation of phosphoglycerate dehydrogenase (PHGDH) is crucial for the apoptotic response upon serine starvation. J Biol Chem 2015; 290: 457-466.

[11] Zhang X and Bai W. Repression of phosphoglycerate dehydrogenase sensitizes triple-negative breast cancer to doxorubicin. Cancer Chemother Pharmacol 2016; 78: 655-659.

[12] Song Z, Feng C, Lu Y, Lin Y and Dong C. PHGDH is an independent prognosis marker and contributes cell proliferation, migration and invasion in human pancreatic cancer. Gene 2018; 642: 43-50.

[13] Samanta D, Park Y, Andrabi SA, Shelton LM, Gilkes DM and Semenza GL. PHGDH expression is required for mitochondrial redox homeostasis, breast cancer stem cell maintenance, and lung metastasis. Cancer Res 2016; 76: 4430-4442.

[14] Xian Y, Zhang S, Wang X, Qin J, Wang W and Wu H. Phosphoglycerate dehydrogenase is a novel predictor for poor prognosis in gastric cancer. Onco Targets Ther 2016; 9: 5553-5560.

[15] Endo F, Nishizuka SS, Kume K, Ishida K, Katagiri H, Ishida K, Sato K, Iwaya T, Koeda K and Wakabayashi G. A compensatory role of NF-kappaB to p53 in response to 5-FU-based chemotherapy for gastric cancer cell lines. PLoS One 2014; 9: e90155.

[16] Cairns RA, Harris IS and Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2011; 11: 85-95.

[17] Ward PS and Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell 2012; 21: 297-308.

[18] DeBerardinis RJ. Serine metabolism: some tumors take the road less traveled. Cell Metab 2011; 14: 285-286.

[19] Zaal EA, Wu W, Jansen G, Zweegman S, Cloos J and Berkers CR. Bortezomib resistance in mul-

tiple myeloma is associated with increased serine synthesis. Cancer Metab 2017; 5: 7.
[20] Jing Z, Heng W, Xia L, Ning W, Yafei Q, Yao Z and Shulan Z. Downregulation of phosphoglycerate dehydrogenase inhibits proliferation and enhances cisplatin sensitivity in cervical adenocarcinoma cells by regulating Bcl-2 and caspase-3. Cancer Biol Ther 2015; 16: 541-548.
[21] Thandapani P and Aifantis I. Apoptosis, up the ante. Cancer Cell 2017; 32: 402-403.
[22] Hassan M, Watari H, AbuAlmaaty A, Ohba Y and Sakuragi N. Apoptosis and molecular targeting therapy in cancer. Biomed Res Int 2014; 2014: 150845.
[23] Fernald K and Kurokawa M. Evading apoptosis in cancer. Trends Cell Biol 2013; 23: 620-633.
[24] Martinou JC and Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. Dev Cell 2011; 21: 92-101.
[25] Manoochehri M, Karbasi A, Bandehpour M and Kazemi B. Down-regulation of BAX gene during carcinogenesis and acquisition of resistance to 5-FU in colorectal cancer. Pathol Oncol Res 2014; 20: 301-307.
[26] Opferman JT. Attacking cancer's Achilles heel: antagonism of anti-apoptotic BCL-2 family members. FEBS J 2016; 283: 2661-2675.
[27] Philchenkov AA. Caspases as regulators of apoptosis and other cell functions. Biochemistry (Mosc) 2003; 68: 365-376.